

High Level of Vector Competence of *Aedes aegypti* and *Aedes albopictus* from Ten American Countries as a Crucial Factor in the Spread of Chikungunya Virus

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ABSTRACT

Chikungunya virus (CHIKV) causes a major public health problem. In 2004, CHIKV began an unprecedented global expansion and has been responsible for epidemics in Africa, Asia, islands in the Indian Ocean region, and surprisingly, in temperate regions, such as Europe. Intriguingly, no local transmission of chikungunya virus (CHIKV) had been reported in the Americas until recently, despite the presence of vectors and annually reported imported cases. Here, we assessed the vector competence of 35 American *Aedes aegypti* and *Aedes albopictus* mosquito populations for three CHIKV genotypes. We also compared the number of viral particles of different CHIKV strains in mosquito saliva at two different times postinfection. Primarily, viral dissemination rates were high for all mosquito populations irrespective of the tested CHIKV isolate. In contrast, differences in transmission efficiency (TE) were underlined in populations of both species through the Americas, suggesting the role of salivary glands in selecting CHIKV for highly efficient transmission. Nonetheless, both mosquito species were capable of transmitting all three CHIKV genotypes, and TE reached alarming rates as high as 83.3% and 96.7% in *A. aegypti* and *A. albopictus* populations, respectively. *A. albopictus* better transmitted the epidemic mutant strain CHIKV_0621 of the East-Central-South African (ECSA) genotype than did *A. aegypti*, whereas the latter species was more capable of transmitting the original ECSA CHIKV_115 strain and also the Asian genotype CHIKV_NC. Therefore, a high risk of establishment and spread of CHIKV throughout the tropical, subtropical, and even temperate regions of the Americas is more real than ever.

IMPORTANCE

Until recently, the Americas had never reported chikungunya (CHIK) autochthonous transmission despite its global expansion beginning in 2004. Large regions of the continent are highly infested with *Aedes aegypti* and *Aedes albopictus* mosquitoes, and millions of dengue (DEN) cases are annually recorded. Indeed, DEN virus and CHIK virus (CHIKV) share the same vectors. Due to a recent CHIK outbreak affecting Caribbean islands, the need for a Pan-American evaluation of vector competence was compelling as a key parameter in assessing the epidemic risk. We demonstrated for the first time that *A. aegypti* and *A. albopictus* populations throughout the continent are highly competent to transmit CHIKV irrespective of the viral genotypes tested. The risk of CHIK spreading throughout the tropical, subtropical, and even temperate regions of the Americas is more than ever a reality. In light of our results, local authorities should immediately pursue and reinforce epidemiological and entomological surveillance to avoid a severe epidemic.

Chikungunya virus (CHIKV) is an alphavirus in the family *Togaviridae* that is transmitted by mosquitoes, mainly *Aedes aegypti* and *Aedes albopictus* within an urban cycle. Since 2004, CHIKV has reemerged in Indian Ocean islands and has caused severe epidemics in several countries in tropical and subtropical regions in Africa and Asia, as well as in temperate Mediterranean areas in Europe (1).

Aedes aegypti is widespread in the Americas, where it is the only confirmed natural dengue virus (DENV) vector (2). Although its geographical distribution is more limited, *A. albopictus* is considered a potential vector in the Americas due to the high level of vector competence of local populations for DENV (3, 4). More than 2 million dengue (DEN) cases are annually reported in the American continent each year (5). The most critical epidemiological situation is that described for South America, which reported more than 1.5 million dengue cases in 2013, with an incidence rate of more than 650 cases/100,000 inhabitants in the South Cone alone (6). Such an epidemiological scenario points to the weak-

ness of mosquito control activities and the high receptivity to introduction and spread of other arboviruses transmitted by both mosquito species like CHIKV in other parts of the continent (1, 7, 8). In fact, as CHIKV and DENV share the same mosquito vector species, epidemic waves caused by both viruses affect the same regions, and human coinfections may occur (9, 10). Moreover, the

Received 5 February 2014 Accepted 17 March 2014

Published ahead of print 26 March 2014

Editor: R. W. Doms

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Supplemental material for this article may be found at <http://dx.doi.org/10.1128/JVI.00370-14>.

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doi:10.1128/JVI.00370-14

intensification of intercontinental travel with recurrent returns of dozens of viremic CHIKV cases from affected areas—which may bypass the surveillance systems due to the clinical similarities to other viruses circulating in the Americas—exemplifies the vulnerability of this continent to CHIKV epidemics (11, 12). Indeed, Brazil, Canada, the United States, French Guiana, and the French West Indies (Guadeloupe and Martinique) have reported several imported cases of CHIKV since its reemergence in 2004 (6, 13).

Intriguingly, until December 2013, autochthonous CHIKV transmission had never been reported in the Americas, a continents in which all of the conditions are apparently suitable for its establishment: (i) the Americas are a virgin continent for CHIKV, (ii) the main mosquito vectors of CHIKV, *A. aegypti* and *A. albopictus*, are present at high densities in most areas, (iii) imported cases are annually reported in periods of high mosquito density and activity, and (iv) temperature and environmental conditions of large tropical and subtropical zones are favorable to mosquito development and activity as well as to viral replication in the vector (11, 14). In early December 2013, two laboratory-confirmed autochthonous CHIKV cases were reported in the French territory of Saint-Martin Island in the Caribbean (6). Very rapidly, an epidemic was established on the island, with almost 2,030 clinical cases and more than 765 confirmed cases, and subsequently, some CHIKV cases were detected in Martinique, Guadeloupe, Saint-Barthelemy, and also French Guiana (15). Therefore, CHIKV is progressively spreading, putting at high epidemic risk the vast areas of the Americas infested with *A. aegypti* and *A. albopictus*.

To achieve efficient transmission, numerous factors regarding the invertebrate and the vertebrate hosts, the virus, and the environmental conditions must ideally converge (16). Concerning the mosquito host, vector competence is considered to be unique and characteristic for each virus-vector pair. Indeed differences of vector competence can be found between different populations belonging to a single insect vector species (17). Vector competence is a quantitative phenotypic parameter controlled by genetic characteristics of both vector and virus, which in turn is influenced by environmental conditions (18–20). Mosquito vector competence to CHIKV and DENV seems to be determined by genotype-by-genotype interactions, in which successful transmission depends on some specific combination of mosquito and viral genetic characteristics (21–26). CHIKV has four major lineages: East-Central-South Africa (ECSA), West Africa, Asian, and the Indian Ocean, a monophyletic lineage descendant from the ECSA group (27). The CHIKV lineages have displayed distinct transmission efficiencies in mosquito vector species and populations (25, 28, 29). Throughout the 2005–2006 CHIKV epidemic in the Indian Ocean region, a CHIKV lineage strain harboring a substitution of an alanine to valine at position 226 of the E1 envelope glycoprotein (E1-A226V) was better transmitted by *A. albopictus* (22, 25, 30). It was later shown that other positions in the E2 glycoprotein exert epistatic effects on the position E1-226V (23, 24), and some substitutions can block the adaptation of E1-226V to *A. albopictus*. These epistatic interactions are lineage specific.

Determination of vector competence of mosquito populations is a key parameter in evaluating the risk of CHIKV transmission and spread. Given the alarming epidemiological situation due to the very recent chikungunya outbreak affecting the Caribbean islands, the need for evaluation of the vector competence of American mosquito populations is compelling. Until now, studies were only limited to mosquitoes from the United States and the French

Caribbean (31–34). With the aim of understanding the factors that may influence CHIKV emergence in the Americas and the risk of a CHIKV epidemic spreading throughout the continent, we carried out a comprehensive Pan-American evaluation of vector competence of 35 *A. aegypti* and *A. albopictus* populations from 10 countries toward three CHIKV isolates belonging to two distinct lineages.

MATERIALS AND METHODS

Ethics statement. The Institut Pasteur animal facility has received accreditation from the French Ministry of Agriculture to perform experiments on live animals in compliance of the French and European regulations on care and protection of laboratory animals. This study was approved by the Institutional Animal Care and Use Committee (IACUC) at the Institut Pasteur. No specific permits were required for the described field studies in locations that are not protected in any way and did not involve endangered or protected species.

Mosquitoes. Thirty-five mosquito populations collected in 10 countries from North, Central, and South America were used: 22 populations of *A. aegypti* and 13 of *A. albopictus* (Fig. 1; Table 1). The mosquitoes were field collected in 2012 with ovitraps (10 to 58 per collection site). The mosquito collection sites were strategically chosen in order to essentially represent the diverse climates, environments, ecotopes, and dengue epidemiological history across the American continent. The field-collected eggs were immersed in water for hatching; larvae were split by 100 to 150 individuals per pan and fed with yeast tablets. Emerging adults were maintained in cages at $28 \pm 1^\circ\text{C}$ with a 14-h-light/10-h-dark cycle, 80% relative humidity, and supplied with a 10% sucrose solution. The F1 generation was used for all infection assays.

Viral strains. Three CHIKV isolates belonging to two distinct lineages were used: two CHIKV isolates from La Réunion and one from New Caledonia. The isolates from La Réunion were the strains (i) CHIKV 05.115 (CHIKV₁₁₅) and (ii) CHIKV 06.21 (CHIKV₀₆₂₁), both isolated in 2005 (35) and provided by the French National Reference Center for Arboviruses at the Institut Pasteur in Paris. The amino acid consensus sequences of these strains differed by only a single substitution: CHIKV₁₁₅ has an alanine at position 226 of the E1 envelope glycoprotein (E1-226A), whereas CHIKV₀₆₂₁ harbors a valine at the same position (E1-226V). It has been shown the E1-A226V substitution is located in a region known to be involved in viral entry via fusion with endosomal membranes (36). Both strains have an alanine at position 98 of the E1 glycoprotein (E1-98A) that has been shown to exert no negative epistatic effects on position E1-226; the position E1-98 is located at the base of the fusion loop and presumably modulates the kinetics of the pH-dependent conformational changes and fusion reaction in the endosomal compartment (37). The viral titer estimated by serial 10-fold dilutions on Vero cells was 10^9 PFU/ml for both CHIKV₁₁₅ and CHIKV₀₆₂₁. Both strains were isolated on *A. albopictus* C6/36 cells from human serum or viral stocks and were produced following three passages on *A. albopictus* C6/36 cells and then harvested and stored at -80°C until used for the mosquito experimental infection assays. The New Caledonia CHIKV strain referenced as NC/2011-568 (CHIKV_{NC}), was isolated in 2011 (28, 37) and provided by the Institut Pasteur of New Caledonia. Phylogenetic analysis using the complete CHIKV_{NC} genome nucleotide sequence demonstrated that CHIKV_{NC} belongs to the Asian lineage, displaying 98.1% nucleotide identity to other isolates of the Asian cluster of CHIKV phylogeny. The CHIKV_{NC} strain has an alanine at position E1-226 (E1-226A) and a threonine at position E1-98 (E1-98T). It has been shown that in contrast with the ECSA genotype, the substitution E1-98T exerts a negative epistatic interaction leading to blocking the ability of Asian CHIKV strains to adapt to *A. albopictus* via the E1-A226V substitution (24). The whole genome sequence of CHIKV_{NC} is available in GenBank under accession no. [HE806461](https://www.ncbi.nlm.nih.gov/nuclseq/HE806461). CHIKV_{NC} 2nd passage was used for the experimental infections of mosquitoes. The titer of CHIKV_{NC} stocks was $10^{8.1}$ PFU/ml.

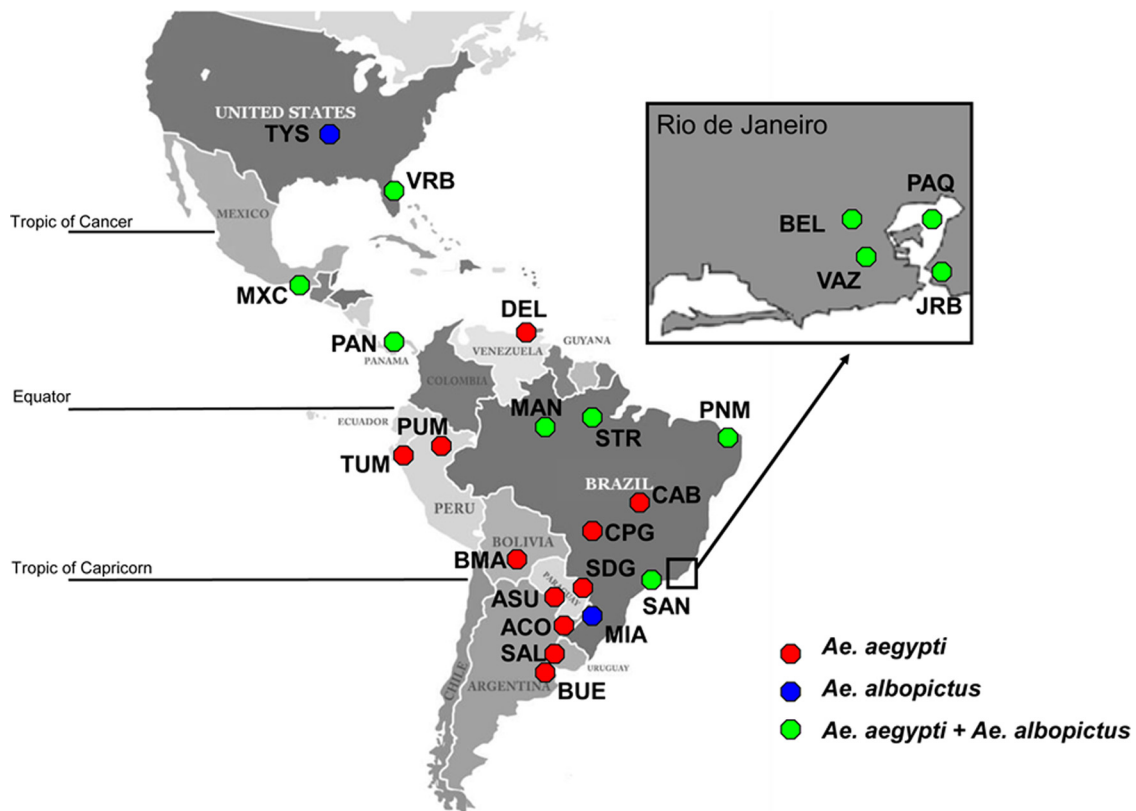


FIG 1 Mosquito populations tested. The color code indicates localities where only *A. aegypti* (red), only *A. albopictus* (blue), and both *A. aegypti* and *A. albopictus* (green) mosquitoes were collected. TYS, Tyson, MO; VRB, Vero Beach, FL; MXC, Chiapas, Mexico; PAN, Panamá, Panamá; DEL, Delta Amacuro, Venezuela; TUM, Tumbes, Peru; PUM, Punchana, Peru; MAN, Manaus, Brazil; STR, Santarém, Brazil; PNM, Parnamirim, Brazil; CAB, Campos Belos, Brazil; CPG, Campo Grande, Brazil; JRB, Jurujuba, Brazil; PAQ, Paquetá, Brazil; VAZ, Vaz Lobo, Brazil; BEL, Belford Roxo, Brazil; SAN, Santos, Brazil; BMA, Monteagudo, Bolivia; SDG, Salto del Guairá, Paraguay; ASU, Asunción, Paraguay; SAL, Salto, Uruguay; MIA, Misiones, Argentina; ACO, Corrientes, Argentina; BUE, Buenos Aires, Argentina.

Mosquito oral infections. Five- to 7-day-old females were fed on an infectious blood meal containing 2 ml of washed rabbit erythrocytes and 1 ml of viral suspension supplemented with a phagostimulant (ATP) at a final concentration of 5 mM. The titer of all performed infectious blood-meals was $10^{7.5}$ PFU/ml. Mosquito feeding was limited to 50 min. After the infectious blood meal, nonengorged females were discarded. Fully engorged females were transferred in cardboard containers and maintained with 10% sucrose at $28^{\circ}\pm 1^{\circ}\text{C}$. All 35 mosquito populations were challenged with the CHIKV_0621 strain (13 *A. albopictus* and 22 *A. aegypti* populations), whereas 22 populations (9 *A. albopictus* and 13 *A. aegypti*) were challenged with the CHIKV_115 strain and 6 populations (3 *A. albopictus* and 3 *A. aegypti*) with CHIKV_NC. Mosquito populations from the same location were simultaneously tested with the CHIKV_0621 and CHIKV_115 strains.

Dissemination and transmission analysis. Batches of ~30 mosquitoes of each combination of mosquito population and virus strain were analyzed at days 7 and 10 postinfection (p.i.) for all CHIKV strains tested. Days p.i. were defined according to the kinetics of CHIKV dissemination and transmission efficiencies (DE and TE, respectively) in *A. albopictus* mosquitoes from Paquetá, Rio de Janeiro, Brazil (maximum at day 7 p.i. and slight decrease by day 10) (Fig. 2). To estimate viral dissemination, heads were removed from mosquitoes and ground in 250 μl of Leibovitz L15 medium (Invitrogen) supplemented with 2% fetal bovine serum (FBS) for further inoculation onto *A. albopictus* C6/36 cell culture in 96-well plates. After incubation at 28°C for 3 days, plates were stained using hyperimmune ascetic fluid specific to CHIKV as the primary antibody. Alexa Fluor 488 goat anti-mouse IgG was used as the second antibody (Life Technologies).

To estimate viral transmission, saliva was collected from individual mosquitoes as described in reference 38. For collection, the wings and legs were removed from each mosquito and the proboscis was inserted into a 20- μl tip containing 5 μl of FBS. After 45 min of salivation, FBS containing saliva was expelled into 45 μl of Leibovitz L15 medium for titration. One limitation of this technique is that the volume of saliva delivered by females could not be estimated.

Dissemination efficiency (DE) corresponds to the proportion of mosquitoes with virus detected in heads among tested ones (i.e., engorged mosquitoes which have survived until the day of examination). Transmission efficiency corresponds to the proportion of mosquitoes with virus in the saliva among tested ones (i.e., surviving females, including females unable to disseminate the virus and those able to disseminate). The number of infectious particles per saliva sample was estimated by titration using a focus fluorescent assay on *A. albopictus* C6/36 cells. Samples were serially diluted and inoculated onto C6/36 cells in 96-well plates, following incubation at 28°C for 3 days, and then the plates were stained as explained above.

Statistical analysis. Statistical analyses were performed with STATISTICA 8 software (StatSoft, Inc., Tulsa, OK). The numbers of infectious particles in saliva were compared using the Kruskal-Wallis test. Dissemination and transmission efficiencies were compared using the chi-square test. Kruskal-Wallis Z multiple-comparison test was used to compare more than 5 dissemination and transmission efficiency rates.

RESULTS

DE. To measure the ability of American *A. aegypti* and *A. albopictus* mosquitoes to allow CHIKV to overcome the midgut barrier,

TABLE 1 Mosquito populations used in this study by country of collection from north to south

Mosquito population	Collection site	Country	Coordinates	Mosquito species used	Climate	Dominant vegetation	Environment	History of dengue incidence ^a
TYs	Tyson, MO	United States	36°31'N, 90°33'W	<i>A. albopictus</i>	Temperate	Temperate grassland	Suburban	F
VRB	Vero Beach, FL	United States	27°35'N, 80°22'W	<i>A. aegypti</i> , <i>A. albopictus</i>	Humid subtropical	Subtropical evergreen forest	Suburban	F
MXC	Tapachula	Mexico	14°53'N, 92°15'W	<i>A. aegypti</i> , <i>A. albopictus</i>	Tropical wet and dry	Tropical deciduous forest	Suburban	M
PAN	Panamá/Colon	Panama	08°59'N, 79°30'W/09°21'N, 79°53'W	<i>A. aegypti</i> , <i>A. albopictus</i>	Tropical wet and dry	Savanna	Urban and suburban	L
DEL	Dela Amacuro, Tucupita	Venezuela	09°03'N, 62°02'W	<i>A. aegypti</i>	Tropical wet and dry	Savanna	Suburban	L
PUM	Punchana, Iquitos	Peru	03°43'S, 73°15'W	<i>A. aegypti</i>	Tropical wet and dry	Amazon forest	Urban	H
TUM	Turnbes, Huauquillas	Peru	03°29'S, 80°15'W	<i>A. aegypti</i>	Arid	Desert	Suburban	L
MAN	Manaus	Brazil	03°06'S, 60°03'W	<i>A. aegypti</i> , <i>A. albopictus</i>	Tropical wet	Amazon forest	Suburban	H
STR	Santarém	Brazil	02°25'S, 54°42'W	<i>A. aegypti</i> , <i>A. albopictus</i>	Tropical wet	Amazon forest	Suburban	M
PNM	Paranamirim	Brazil	05°54'S, 35°16'W	<i>A. aegypti</i> , <i>A. albopictus</i>	Semi-arid	Transitional tropical rainforest	Suburban	H
CAB	Campos Belos	Brazil	13°02'S, 46°46'W	<i>A. aegypti</i>	Tropical wet and dry	Savanna	Urban	L
BEL	Belford Roxo, Rio de Janeiro	Brazil	22°45'S, 43°24'W	<i>A. aegypti</i> , <i>A. albopictus</i>	Tropical wet and dry	Atlantic rain forest	Suburban	H
VAZ	Vaz Lobo, Rio de Janeiro	Brazil	22°51'S, 43°19'W	<i>A. aegypti</i> , <i>A. albopictus</i>	Tropical wet and dry	Atlantic rain forest	Urban	H
JRB	Jurujuba, Rio de Janeiro	Brazil	22°55'S, 43°07'W	<i>A. aegypti</i> , <i>A. albopictus</i>	Tropical wet and dry	Atlantic rain forest	Suburban	L
PAQ	Paqueta, Rio de Janeiro	Brazil	22°45'S, 43°06'W	<i>A. aegypti</i> , <i>A. albopictus</i>	Tropical wet and dry	Atlantic rain forest	Suburban island	M
SAN	Santos	Brazil	23°57'S, 46°20'W	<i>A. aegypti</i> , <i>A. albopictus</i>	Tropical wet and dry	Atlantic rain forest	Suburban	M
CPG	Campo Grande	Brazil	20°27'S, 54°37'W	<i>A. aegypti</i>	Tropical wet and dry	Savanna	Urban	H
BMA	Monteagudo	Bolivia	19°48'S, 63°57'W	<i>A. aegypti</i>	Tropical wet and dry	Mountain forest	Urban	L
ASU	Asunción	Paraguay	25°18'S, 57°37'W	<i>A. aegypti</i>	Tropical wet and dry	Chaco	Urban	M
SDG	Salto del Guairá	Paraguay	24°03'S, 54°18'W	<i>A. aegypti</i>	Humid subtropical	Savanna	Suburban	L
MIA	Misiones	Argentina	25°36'S, 54°34'W	<i>A. albopictus</i>	Humid subtropical	Paranaense forest	Rural	L
ACO	Corrientes	Argentina	27°28'S, 58°50'W	<i>A. aegypti</i>	Humid subtropical	Humid Chaco	Urban	M
BUE	Buenos Aires	Argentina	34°35'S, 58°22'W	<i>A. aegypti</i>	Temperate	Pampas	Urban	L
SAL	Salto	Uruguay	31°23'S, 57°58'W	<i>A. aegypti</i>	Temperate	Pampa	Urban	F

^a F, free; L, low; M, medium; H, high.

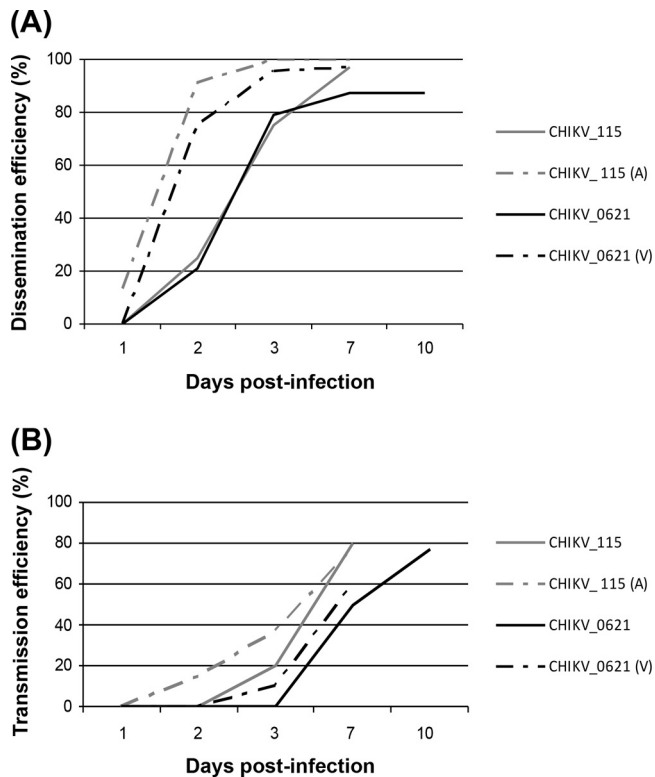


FIG 2 Dissemination (A) and transmission (B) efficiencies of two CHIKV isolates and two clones of the respective viral isolates in *A. albopictus* mosquitoes from Paqueta, Rio de Janeiro, Brazil. At days 1, 2, 3, 7, and 10 after an infectious blood meal, mosquitoes were sacrificed, and heads and saliva were collected for determination of their infectious status. Mosquito heads were individually ground in 250 μ l Leibovitz L15 medium supplemented with 4% FBS, following inoculation onto an *A. albopictus* C6/36 cell monolayer in 96-well plates and incubation at 28°C for 3 days. Plates were fixed with 3.6% formaldehyde, washed three times with PBS, and analyzed by indirect immunofluorescence assay (IFA). For saliva collection, each mosquito had the wings and legs removed, and the proboscis was inserted into a 20- μ l tip containing 5 μ l of FBS. After 45 min of salivation, FBS containing saliva was expelled into 45 μ l of Leibovitz L15 medium and inoculated onto an *A. albopictus* C6/36 cell monolayer in 96-well plates. Plates were incubated and stained (IFA) as described in Materials and Methods. Dissemination efficiency corresponds to the proportion of mosquito females with disseminated virus in the head among the tested mosquitoes. Transmission efficiency corresponds to the proportion of mosquitoes with infectious saliva among the tested mosquitoes. CHIKV_0621 is a strain isolated from La Réunion (E1-226V substitution), CHIKV_115 is a strain isolated from La Réunion (E1-226A), CHIKV_0621 (V) is a clone corresponding to a single virus isolated from CHIKV_0621, and CHIKV_115 (A) is a clone corresponding to a single virus isolated from CHIKV_115. Clones were provided by C. Arias-Goeta, Institut Pasteur, Paris, France.

dissemination efficiency (DE) was assessed for each pairing of mosquito population and virus strain at days 7 and 10 p.i. (Tables 2 and 3).

All *A. aegypti* and *A. albopictus* populations showed similar DE values at days 7 and 10 p.i. for the three CHIKV isolates (chi-square test, $P > 0.05$). For CHIKV_0621, DE at day 7 p.i. ranged from 60% to 100% for *A. albopictus* and from 93.3% to 100% for *A. aegypti*. For CHIKV_115, DE at day 7 varied from 66.7% to 96.9% for *A. albopictus* and from 96.6% to 100% for *A. aegypti*, while for CHIKV_NC, DE ranged from 90% to 96.7% for *A. albopictus* and from 96.9% to 100% for *A. aegypti*. The *A. aegypti*

populations tested displayed similar DE values of around 100% for the three CHIKV isolates (chi-square test, $P > 0.05$). Likewise, DE values obtained for *A. albopictus* were extensively high, although rates were significantly heterogeneous for CHIKV_0621 (chi-square test, $P < 0.05$) and CHIKV_115 (chi-square test, $P < 0.05$). Thus, when comparing DE values for a given virus between the two mosquito species sampled in a same location, no significant difference was found, except for MXC in Mexico when infected with CHIKV_0621 (chi-square test, $P < 0.05$) and CHIKV_115 (chi-square test, $P < 0.05$) and for VRB in the United States when infected with CHIKV_115 (chi-square test, $P < 0.05$). In these last three cases, *A. aegypti* exhibited a higher DE than *A. albopictus* collected in the same site whatever the viral strain. In addition, no difference was observed in DE values between the three *A. aegypti* and *A. albopictus* populations challenged with the CHIKV_NC isolate (chi-square test, $P > 0.05$).

TE. In order to determine the ability of American *A. aegypti* and *A. albopictus* mosquitoes to sustain CHIKV transmission, we assessed transmission efficiency (TE) at days 7 and 10 p.i. Only TE values at day 7 p.i. are presented in Fig. 3 and 4. (For TE values at day 10 p.i., see Table S1 in the supplemental material.) The TE values obtained for *A. aegypti* and *A. albopictus* were highly heterogeneous and lower than the DE values.

When mosquitoes were exposed to CHIKV_0621, TE values ranged from 13.3% to 96.7% at day 7 p.i. and 6.7% to 85.2% at day 10 p.i. *A. albopictus* better transmitted CHIKV_0621 than *A. aegypti* at day 7 p.i. (mean \pm confidence interval [CI], 44.7% \pm 7.8% for *A. aegypti* and 55.8% \pm 12.3% for *A. albopictus*) and at day 10 p.i. (mean \pm CI, 33.1% \pm 6.2% for *A. aegypti* and 55.5% \pm 12.0% for *A. albopictus*). Within the same mosquito species, TE values were significantly different (chi-square test, $P < 0.05$) at days 7 and 10 p.i. When considering each of the 10 populations where the two species coexist (VRB, MXC, PAN, MAN, PNM, JRB, PAQ, VAZ, BEL, and SAN), *A. albopictus* exhibited a higher TE than *A. aegypti* when infected with CHIKV_0621, except for the VRB population from Florida (Fig. 3 and 4; see Table S1 in the supplemental material).

When mosquitoes were infected with CHIKV_115, TE values comprised between 11.1% and 82.1% at day 7 p.i. and 10% and 76.7% at day 10 p.i. *A. aegypti* better transmitted CHIKV_115 than *A. albopictus* at day 7 p.i. (mean \pm CI, 49.5% \pm 10.3% for *A. aegypti* and 49.5% \pm 13.6% for *A. albopictus*). Within the same mosquito species, TE values were significantly different (chi-square test, $P < 0.05$) at days 7 and 10 p.i. When considering each of the four populations where the two species coexist (VRB, MXC, PAN, and PAQ), one species did not present a clear-cut advantage over the other to transmit CHIKV_115 (Fig. 3 and 4; see Table S1 in the supplemental material).

Interestingly, among the eight *A. albopictus* populations simultaneously challenged with CHIKV_0621 and CHIKV_115, four showed unexpected lower TE for CHIKV_115 and one displayed equal rates (Fig. 3; see Table S1 in the supplemental material). Remarkably, TE rates were heterogeneous even between *A. albopictus* populations geographically close, i.e., from Rio de Janeiro, Brazil (JRB, PAQ, BEL, and VAZ), when exposed to the same CHIKV_0621 isolate (Fig. 3 and 4).

Finally, when mosquitoes were exposed to the CHIKV_NC strain, TE values varied from 30% to 83.3% at day 7 p.i. and from 26.7% to 53.3% at day 10 p.i. *A. aegypti* better transmitted CHIKV_NC than *A. albopictus* at day 7 p.i. (mean \pm CI, 64.5% \pm

TABLE 2 Dissemination efficiency of three CHIKV isolates in 22 *A. aegypti* and 13 *A. albopictus* populations from 10 American countries at day 7 postinfection

Country	Mosquito population ^a	% dissemination efficiency (no. of mosquitoes) ^b					
		CHIKV_0621		CHIKV_115		CHIKV_NC	
		<i>A. aegypti</i>	<i>A. albopictus</i>	<i>A. aegypti</i>	<i>A. albopictus</i>	<i>A. aegypti</i>	<i>A. albopictus</i>
United States	TYS	ND	96.7 (30)	ND	83.3 (30)	ND	ND
	VRB	100 (30)	93.3 (30)	100 (18)	73.3 (30)*	ND	ND
Mexico	MXC	96.7 (30)	73.3 (30)*	96.7 (30)	66.7 (30)*	ND	ND
Panama	PAN	96.7 (30)	96.7 (30)	96.7 (30)	93.3 (30)	100 (30)	96.7 (30)
Venezuela	DEL	100 (23)	ND	100 (28)	ND	ND	ND
Peru	TUM	100 (30)	ND	ND	ND	ND	ND
	PUM	100 (30)	ND	100 (29)	ND	ND	ND
Brazil	MAN	100 (30)	96.7 (30)	ND	90.3 (31)	100 (30)	90 (30)
	STR	100 (30)	100 (30)	ND	88.4 (26)	ND	ND
	PNM	100 (30)	93.3 (30)	ND	ND	ND	ND
	CAB	100 (30)	ND	ND	ND	ND	ND
	CPG	100 (30)	ND	100 (30)	ND	ND	ND
	JRB	100 (30)	100 (30)	100 (30)	ND	ND	ND
	PAQ	100 (30)	87.1 (31)	100 (30)	96.9 (29)	ND	ND
	VAZ	100 (30)	91.3 (23)	ND	ND	ND	ND
	BEL	100 (30)	90.9 (22)	ND	ND	ND	ND
	SAN	93.3 (30)	100 (30)	ND	87.5 (8)	ND	ND
Bolivia	BMA	100 (30)	ND	100 (30)	ND	ND	ND
Paraguay	SDG	100 (30)	ND	ND	ND	ND	ND
	ASU	100 (30)	ND	96.7 (30)	ND	ND	ND
Uruguay	SAL	100 (30)	ND	100 (30)	ND	ND	ND
Argentina	MIA	ND	60 (30)	ND	66.7 (26)	ND	93.3 (30)
	ACO	100 (30)	ND	100 (30)	ND	ND	ND
	BUE	100 (30)	ND	96.6 (29)	ND	96.9 (33)	ND

^a Mosquito populations (from north to south): TYS, Tyson, MO; VRB, Vero Beach, FL; MXC, Chiapas, Mexico; PAN, Panamá, Panama; DEL, Delta Amacuro, Venezuela; TUM, Tumbes, Peru; PUM, Punchana, Peru; MAN, Manaus, Brazil; STR, Santarém, Brazil; PNM, Parnamirim, Brazil; CAB, Campos Belos, Brazil; CPG, Campo Grande, Brazil; JRB, Jurujuba, Brazil; PAQ, Paquetá, Brazil; VAZ, Vaz Lobo, Brazil; BEL, Belford Roxo, Brazil; SAN, Santos, Brazil; BMA, Monteagudo, Bolivia; SDG, Salto del Guairá, Paraguay; ASU, Asunción, Paraguay; SAL, Salto, Uruguay; MIA, Misiones, Argentina; ACO, Corrientes, Argentina; BUE, Buenos Aires, Argentina.

^b Dissemination efficiency corresponds to the proportion of mosquitoes with disseminated virus in heads among tested ones. The numbers of analyzed mosquitoes are shown in parentheses. The titer of infectious blood meals was 10^{7-5} PFU/ml. CHIKV_0621 was isolated from La Réunion (ECSA genotype, E1-226V and E1-98A substitutions), CHIKV_115 was isolated from La Réunion (ECSA genotype, E1-226A and E1-98A substitutions), and CHIKV_NC was isolated from New Caledonia (Asian genotype, E1-226A and E1-98T substitutions). ND, not determined. Statistically significant differences in dissemination efficiency between the two mosquito species for a given virus are shown by asterisks ($P < 0.05$).

20.7% for *A. aegypti* and $48.9\% \pm 25.1\%$ for *A. albopictus*). Within the same mosquito species, TE values were significantly different (chi-square test, $P < 0.05$) at day 7 and not at day 10 p.i. (chi-square test, $P > 0.05$) (see Table S1 in the supplemental material).

We also found that 23% to 56% of mosquitoes collected in temperate regions, *A. albopictus* TYS (Tyson, MO) and *A. aegypti* SAL (Salto, Uruguay) and BUE (Buenos Aires, Argentina) were able to efficiently transmit CHIKV_0621. Moreover, *A. aegypti* mosquitoes from the last two sites of the Southern Cone were also competent to efficiently transmit CHIKV_0115 and CHIKV_NC at day 7 p.i., respectively (SAL, 70% for CHIKV_115; BUE, 48.3% for CHIKV_115 and 63.6% for CHIKV_NC).

Intensity of transmission. The intensity of viral transmission can be calculated by estimating the viral load in saliva collected from mosquitoes. When infected with the CHIKV_0621 isolate,

the number of viral particles in saliva ranged from 0.4 to $4.4 \log_{10}$ particles for *A. albopictus* and from 0.4 to $5.1 \log_{10}$ particles for *A. aegypti*. Concerning mosquitoes infected with the CHIKV_115 isolate, the number of viral infectious particles varied from 0.4 to $4.7 \log_{10}$ for *A. albopictus* and from 0.4 to $5.0 \log_{10}$ for *A. aegypti*. For mosquitoes exposed to CHIKV_NC, the viral load in saliva ranged from 0.4 to $2.9 \log_{10}$ particles for *A. albopictus* and from 0.4 to $4.2 \log_{10}$ particles for *A. aegypti* (Fig. 5). Viral loads of the three tested CHIKV strains were equivalent in *A. aegypti* populations, whereas *A. albopictus* displayed a slightly lower titer when challenged with CHIKV_NC in comparison to CHIKV_0621 and CHIKV_115, both at day 7 p.i. Viral loads were highly heterogeneous between individuals belonging to the same population and infected with a given viral strain, but the means calculated for each mosquito population were roughly similar overall. Indeed, when

TABLE 3 Dissemination efficiency of three CHIKV isolates in 22 *A. aegypti* and 13 *A. albopictus* populations from 10 American countries at day 10 postinfection

Country	Mosquito population ^a	% dissemination efficiency (no. of mosquitoes) ^b					
		CHIKV_0621		CHIKV_115		CHIKV_NC	
		<i>A. aegypti</i>	<i>A. albopictus</i>	<i>A. aegypti</i>	<i>A. albopictus</i>	<i>A. aegypti</i>	<i>A. albopictus</i>
United States	TYS	ND	93.3 (30)	ND	63.6 (11)	ND	ND
	VRB	100 (30)	85.7 (7)*	ND	96.7 (30)	ND	ND
Mexico	MXC	93.3 (30)	70.0 (30)*	100 (30)	53.3 (30)***	ND	ND
Panama	PAN	100 (30)	96.7 (30)	96.7 (30)	83.3 (30)	100 (30)	96.7 (30)
Venezuela	DEL	100 (10)	ND	100 (15)	ND	ND	ND
Peru	TUM	100 (30)	ND	ND	ND	ND	ND
	PUM	100 (29)	ND	100 (30)	ND	ND	ND
Brazil	MAN	100 (30)	100 (36)	ND	97.1 (34)	100 (30)	93.3 (30)
	STR	100 (30)	100 (20)	ND	ND	ND	ND
	PNM	100 (30)	90 (30)	ND	ND	ND	ND
	CAB	100 (30)	ND	ND	ND	ND	ND
	CPG	100 (30)	ND	100 (29)	ND	ND	ND
	JRB	100 (30)	100 (30)	100 (30)	ND	ND	ND
	PAQ	100 (30)	87.5 (32)*	100 (30)	ND	ND	ND
	VAZ	96.7 (30)	100 (32)	ND	ND	ND	ND
	BEL	100 (30)	88.9 (27)	ND	ND	ND	ND
	SAN	100 (29)	100 (30)	ND	ND	ND	ND
Bolivia	BMA	100 (30)	ND	100 (30)	ND	ND	ND
Paraguay	SDG	100 (30)	ND	ND	ND	ND	ND
	ASU	100 (30)	ND	93.3 (30)	ND	ND	ND
Uruguay	SAL	100 (30)	ND	100 (30)	ND	ND	ND
Argentina	MIA	ND	93.3 (30)	ND	80 (30)	ND	96.7 (30)
	ACO	100 (30)	ND	96.7 (30)	ND	ND	ND
	BUE	96.7 (30)	ND	100 (30)	ND	90 (30)	ND

^a Mosquito populations (from north to south): TYS, Tyson, MO; VRB, Vero Beach, FL; MXC, Chiapas, Mexico; PAN, Panamá, Panama; DEL, Delta Amacuro, Venezuela; TUM, Tumbes, Peru; PUM, Punchana, Peru; MAN, Manaus, Brazil; STR, Santarém, Brazil; PNM, Parnamirim, Brazil; CAB, Campos Belos, Brazil; CPG, Campo Grande, Brazil; JRB, Jurujuba, Brazil; PAQ, Paquetá, Brazil; VAZ, Vaz Lobo, Brazil; BEL, Belford Roxo, Brazil; SAN, Santos, Brazil; BMA, Monteagudo, Bolivia; SDG, Salto del Guairá, Paraguay; ASU, Asunción, Paraguay; SAL, Salto, Uruguay; MIA, Misiones, Argentina; ACO, Corrientes, Argentina; BUE, Buenos Aires, Argentina.

^b Dissemination efficiency corresponds to the proportion of mosquitoes with disseminated virus in heads among tested ones. Numbers of analyzed mosquitoes are shown in parentheses. CHIKV_0621 was isolated from La Réunion (ECSA genotype, E1-226V and E1-98A substitutions), CHIKV_115 was isolated from La Réunion (ECSA genotype, E1-226A and E1-98A substitutions), and CHIKV_NC was isolated from New Caledonia (Asian genotype, E1-226A and E1-98T substitutions). ND, not determined. Statistically significant differences in dissemination efficiencies between the two mosquito species for a given virus are shown by asterisks (*, $P < 0.05$; ***, $P < 0.001$).

comparing viral loads in saliva between mosquito strains for a given virus at days 7 and 10 p.i. (Fig. 5; see Fig. S1 in the supplemental material), no significant differences were found for either *A. aegypti* or *A. albopictus* (Kruskal-Wallis test, $P > 0.05$), except for *A. albopictus* challenged with CHIKV_115.

DISCUSSION

All 35 populations of *A. aegypti* and *A. albopictus* mosquitoes collected throughout the Americas were susceptible to CHIKV infection by all three tested genotypes. Thus, temperate as well as tropical and subtropical North, Central, and South American *Aedes* mosquitoes are efficient CHIKV vectors. *A. albopictus* better transmitted the epidemic CHIKV_0621 strain isolated on La Réunion Island in 2006 (35) than *A. aegypti*, whereas the latter species was more capable at transmitting the original strain,

CHIKV_115, both belonging to the ECSA genotype (39). The Asian genotype represented by the CHIKV_NC strain (28) was better transmitted by *A. aegypti*, although it was also efficiently transmitted by *A. albopictus*.

Most American *Aedes* mosquitoes are highly susceptible to CHIKV. More than 60% of mosquitoes per population were able to disseminate CHIKV after crossing the midgut barrier (i.e., entry in epithelial cells, viral replication, and release of virions from the midgut basal lamina). Thus, after being ingested with a blood meal provided at a titer of $10^{7.5}$ PFU/ml, CHIKV succeeded in disseminating within the mosquito hemocoel, which is an essential prerequisite for transmission. It has been shown that a titer of $\sim 10^4$ PFU/ml in monkeys was sufficient to infect mosquitoes (40). CHIKV transmission was highly heterogeneous in American mosquitoes, ranging from 11.1% to 96.7% at day 7 p.i. when consid-

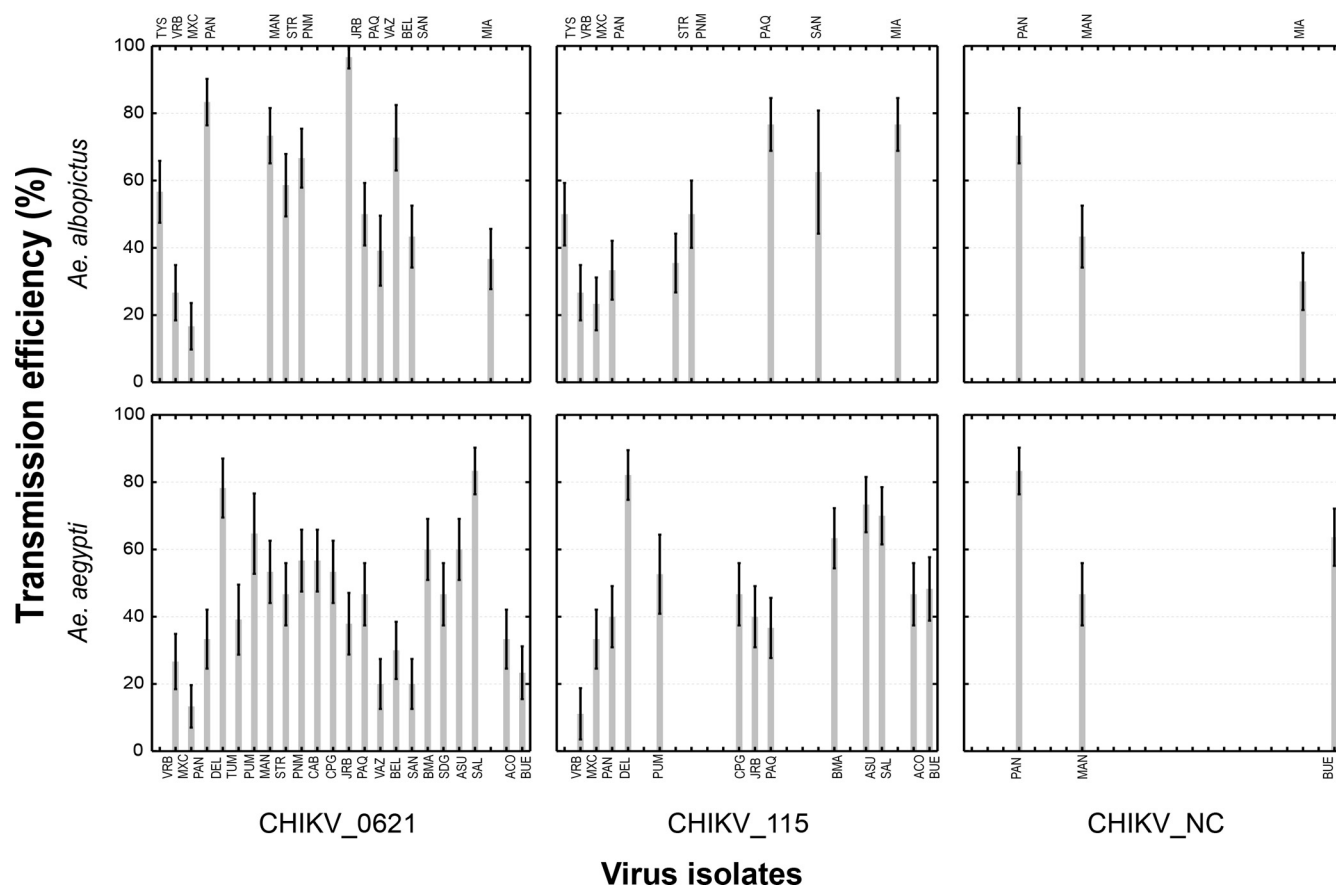


FIG 3 Transmission efficiency of three CHIKV isolates in 35 *A. albopictus* and *A. aegypti* populations from 10 American countries at day 7 postinfection. After an infectious blood meal, mosquitoes were sacrificed, and saliva was collected from individual mosquitoes and titrated by focus fluorescent assay on *A. albopictus* C6/36 cells to determine infectious status. Transmission efficiency corresponds to the proportion of mosquitoes with infectious saliva among those tested. Viral strains are as follows: CHIKV_0621 was isolated from La Réunion (ECSA genotype, E1-226V and E1-98A substitutions), CHIKV_115 was isolated from La Réunion (ECSA genotype, E1-226A and E1-98A substitutions), and CHIKV_NC was isolated from New Caledonia (Asian genotype, E1-226A and E1-98T substitutions). Mosquito populations are as follows (from north to south): TYS, Tyson, MO; VRB, Vero Beach, FL; MXC, Chiapas, Mexico; PAN, Panamá, Panamá; DEL, Delta Amacuro, Venezuela; TUM, Tumbes, Peru; PUM, Punchana, Peru; MAN, Manaus, Brazil; STR, Santarém, Brazil; PNM, Parnamirim, Brazil; CAB, Campos Belos, Brazil; CPG, Campo Grande, Brazil; JRB, Jurujuba, Brazil; PAQ, Paquetá, Brazil; VAZ, Vaz Lobo, Brazil; BEL, Belford Roxo, Brazil; SAN, Santos, Brazil; BMA, Monteagudo, Bolivia; SDG, Salto del Guairá, Paraguay; ASU, Asunción, Paraguay; SAL, Salto, Uruguay; MIA, Misiones, Argentina; ACO, Corrientes, Argentina; and BUE, Buenos Aires, Argentina. Error bars show 95% confidence intervals.

ering all CHIKV strains. It should be underlined that we are not able to provide a control of salivation, and we hypothesize that a CHIKV-negative saliva sample did not correspond to mosquitoes unable to salivate but to mosquitoes delivering noninfected saliva. As expected from previous studies (22, 25, 30, 41), *A. albopictus* better transmitted the epidemic strain CHIKV_0621 of the ECSA genotype than *A. aegypti*, even in cases where both mosquito species cohabit. *A. aegypti* transmitted preferentially CHIKV_115 and also the Asian genotype CHIKV_NC in accordance with previous findings (28). CHIKV Asian strains have a particular E1-98T substitution that constrains CHIKV adaptation to *A. albopictus* via E1-A226V mutation (24). *A. aegypti* mosquitoes are more abundant in the Americas than *A. albopictus* mosquitoes, and the E1-98T substitution of CHIKV viral strains does not have a negative effect on CHIKV interaction with *A. aegypti*. Thus, CHIKV Asian strains together with the CHIKV ECSA strains represent a real danger to the Americas. Intriguingly, the CHIKV strain isolated during the last outbreak in the Caribbean also belongs to the Asian genotype (42) primarily transmitted in the past by *A. aegypti*. Al-

though the intensity of transmission is highly variable between mosquitoes, the mean numbers of viral particles delivered by mosquitoes were quite similar for each combination of mosquito strain and viral strain.

Mosquitoes collected in tropical Latin America, Panama, Venezuela, Brazil, Bolivia, Paraguay, Argentina, and Uruguay showed the highest transmission efficiency, with up to 10,000 viral particles detected in mosquito saliva. Interestingly, mosquitoes from the main Brazilian city of Rio de Janeiro showed high transmission efficiencies. For example, 96.7% of *A. albopictus* JRB mosquitoes were able to transmit CHIKV_0621 (see Table S1 in the supplemental material). Moreover, the extrinsic incubation periods of CHIKV (i.e., the time necessary for the virus to be detected in saliva ready for transmission after being ingested with the blood meal [43]), in both mosquito species are quite short (38). Indeed, an *A. albopictus* population from Rio de Janeiro (PAQ) was able to transmit infectious viral particles as rapidly as 2 days p.i. (Fig. 2). Therefore, the risk of CHIKV establishment in densely populated cities, such as Rio

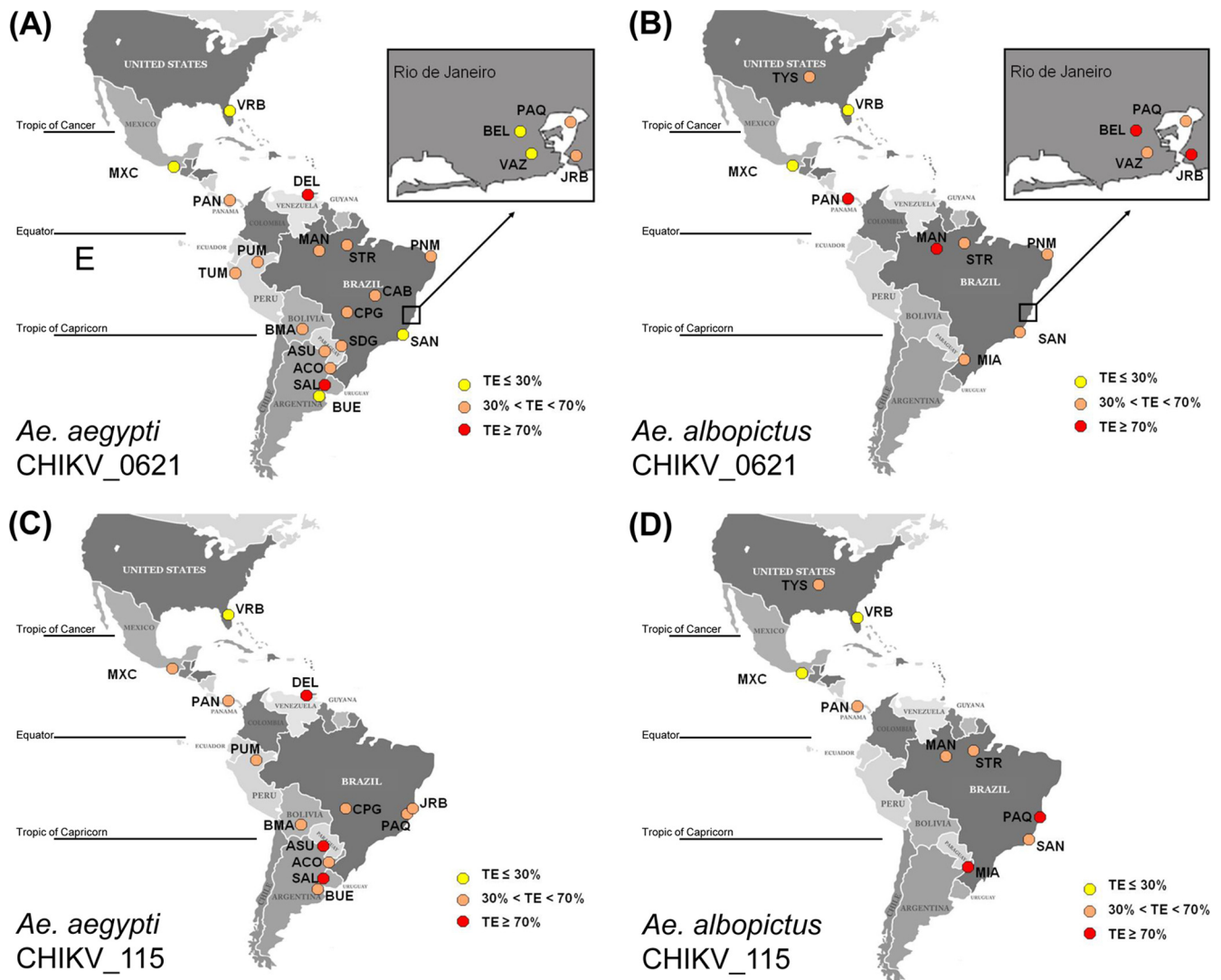


FIG 4 Transmission efficiency of CHIKV_0621 and CHIKV_115 isolates in 35 *A. aegypti* and *A. albopictus* populations from 10 American countries at day 7 postinfection. Transmission efficiency corresponds to the proportion of mosquitoes with infectious saliva among those tested. The color code indicates different degrees of transmission efficiency (TE): yellow, mosquito strains with TE ≤ 30% (low TE); pale orange, strains with 30% < TE < 70% (moderate TE); red, strains with TE ≥ 70% (high TE). The viral strains are as follows: CHIKV_0621 was isolated from La Réunion (ECSA genotype, E1-226V substitution) and CHIKV_115 isolated from La Réunion (ECSA genotype, E1-226A substitution). The mosquito populations are as follows (from north to south): TYS, Tyson, MO; VRB, Vero Beach, FL; MXC, Chiapas, Mexico; PAN, Panamá, Panama; DEL, Delta Amacuro, Venezuela; TUM, Tumbes, Peru; PUM, Punchana, Peru; MAN, Manaus, Brazil; STR, Santarém, Brazil; PNM, Parnamirim, Brazil; CAB, Campos Belos, Brazil; CPG, Campo Grande, Brazil; JRB, Jurujuba, Brazil; PAQ, Paquetá, Brazil; VAZ, Vaz Lobo, Brazil; BEL, Belford Roxo, Brazil; SAN, Santos, Brazil; BMA, Monteagudo, Bolivia; SDG, Salto del Guairá, Paraguay; ASU, Asunción, Paraguay; SAL, Salto, Uruguay; MIA, Misiones, Argentina; ACO, Corrientes, Argentina; BUE, Buenos Aires, Argentina.

de Janeiro, hosting more than 6 million people and infested by anthropophilic *Aedes* mosquitoes, should be considered very high.

Mosquitoes from temperate regions of the Americas are potentially capable of sustaining CHIKV transmission. The ability of CHIKV to extend its natural range of distribution to include temperate regions was exemplified by the Italian outbreak in 2007 and the French local, autochthonous cases in 2010 (44, 45). In the Americas, more than 100 imported CHIKV cases were detected in the United States between 1995 and 2009 (11). Some of them developed a viremia high enough to infect mosquitoes. We found that 56.7% of *A. albopictus* TYS mosquitoes from Tyson, MO, and 83.3% of *A. aegypti* SAL mosquitoes from Salto, Uruguay, were

able to transmit CHIKV_0621 at day 7 p.i. (see Table S1 in the supplemental material). Transmission efficiencies were lower for *A. aegypti* BUE from Buenos Aires, Argentina (i.e., 23.3%) (Fig. 3; see Table S1), but were higher when infected with the CHIKV_NC Asian genotype (i.e., 63.6%) (Fig. 3; see Table S1). Therefore, the establishment of CHIKV in temperate American countries is not simply a fiction, even if less than 30% of both mosquito species collected in the southern part of the United States (VRB from Florida) were able to transmit CHIKV_0621. It has been found that *A. albopictus* mosquitoes from Florida are more competent vectors of CHIKV than *A. aegypti* (31–33). Outbreaks of DENV, also transmitted by *Aedes* mosquitoes, have occurred in Texas and Florida in the past few years (46), reinforcing the risk of epidemics

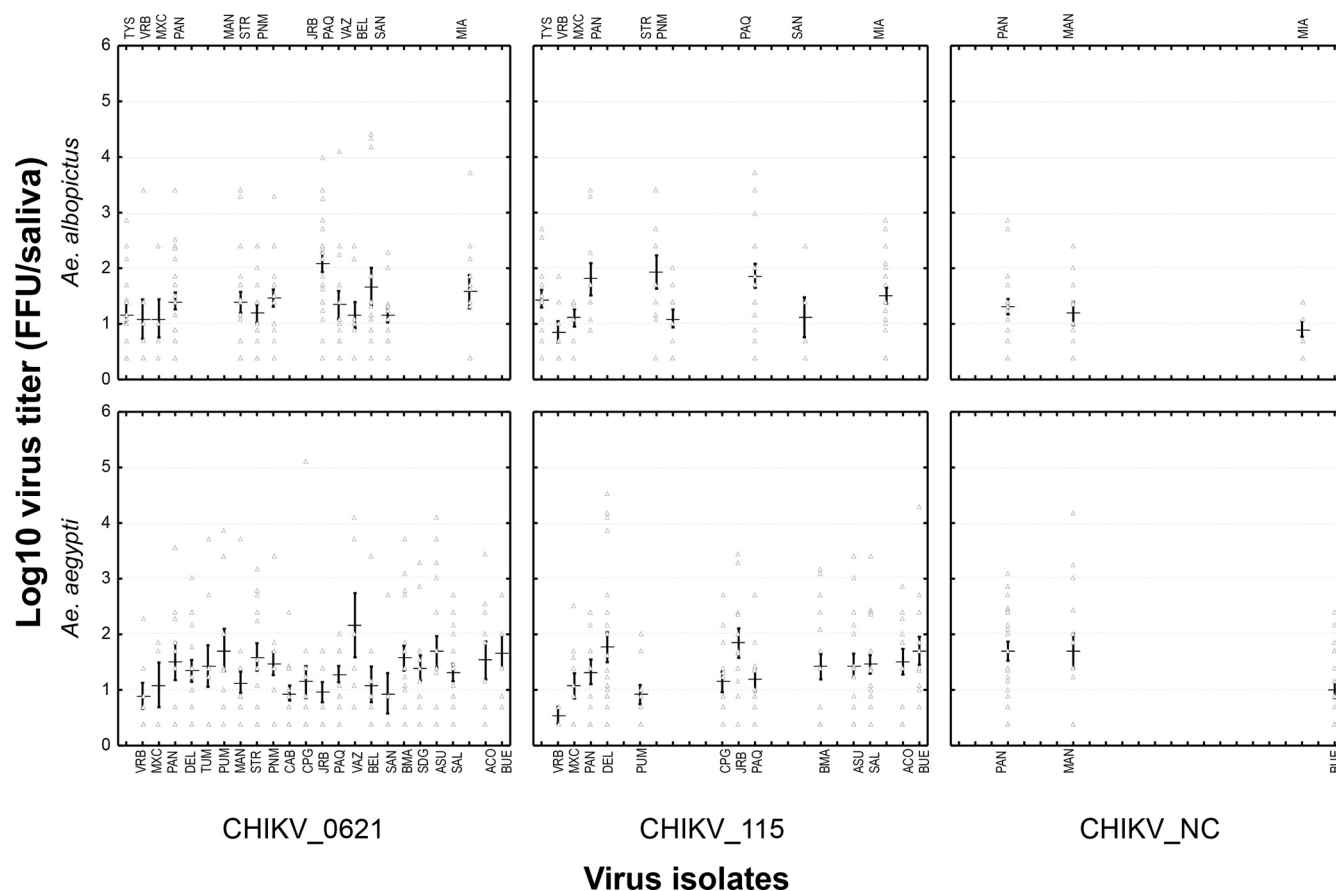


FIG 5 Viral loads of three CHIKV isolates in saliva of *A. albopictus* and *A. aegypti* mosquitoes from 35 populations from the Americas at day 7 postinfection. At day 7 after an infectious blood meal, mosquitoes were sacrificed, and saliva was collected from individual mosquitoes and titrated by focus fluorescent assay on *A. albopictus* C6/36 cells. The viral strains are as follows: CHIKV_0621 was isolated from La Réunion (ECSA genotype, E1-226V and E1-98A substitutions), CHIKV_115 was isolated from La Réunion (ECSA genotype, E1-226A and E1-98A substitutions), and CHIKV_NC was isolated from New Caledonia (Asian genotype, E1-226A and E1-98T substitutions). The mosquito populations are as follows (from north to south): TYR, Tyson, MO; VRB, Vero Beach, FL; MXC, Chiapas, Mexico; PAN, Panamá, Panama; DEL, Delta Amacuro, Venezuela; TUM, Tumbes, Peru; PUM, Punchana, Peru; MAN, Manaus, Brazil; STR, Santarém, Brazil; PNM, Parnamirim, Brazil; CAB, Campos Belos, Brazil; CPG, Campo Grande, Brazil; JRB, Jurujuba, Brazil; PAQ, Paquetá, Brazil; VAZ, Vaz Lobo, Brazil; BEL, Belford Roxo, Brazil; SAN, Santos, Brazil; BMA, Monteagudo, Bolivia; SDG, Salto del Guairá, Paraguay; ASU, Asunción, Paraguay; SAL, Salto, Uruguay; MIA, Misiones, Argentina; ACO, Corrientes, Argentina; BUE, Buenos Aires, Argentina. Error bars refer to the standard error of the mean titer for each pairing of mosquito population and virus strain.

due to imported arboviruses in the United States. Local transmission of CHIKV could be maintained if the virus is introduced in the right place at the right time. Taken together, these findings underline the high variation of susceptibility to CHIKV of American mosquitoes, calling for the inclusion of other factors (biological and environmental) in assessing potential risk of transmission (47). Moreover, the mosquitoes' genetic structure should be promptly investigated. Phylogenetic analysis of both mosquito species should bring additional information on the colonization history of *A. aegypti* and *A. albopictus* in the different countries of the Americas (48, 49). *A. aegypti* was most likely introduced in North America during the slave trade (50), while *A. albopictus* was established in 1985 in the United States (51), probably introduced in shipments of used tires from Japan (52), and in Brazil in 1986 (53), probably arriving from tropical Asia (52).

The fear becomes a reality. Still absent until very recently, CHIKV was detected for the first time in the Americas in late December 2013. Currently, among the 2,030 suspected CHIKV cases from the island of Saint-Martin in the Caribbean, more than

765 were confirmed positive for CHIKV by serology (15). The virus then spread to neighboring islands: Saint-Barthelemy with 380 cases, Martinique with 3,940 cases, and Guadeloupe with 1,460 cases. Until now, 10 autochthonous cases have been reported in French Guiana, which maintains a daily air link with the two other French Overseas Territories of Guadeloupe and Martinique. We previously showed that *A. aegypti* mosquitoes from French Guiana and French West Indies were highly competent to disseminate CHIKV and that mosquito populations collected in dense housing environments exhibited the highest susceptibility (34). Thus, the risk of CHIKV spread and establishment is real and should concern all areas in the Americas where the vector mosquitoes are present.

Cocirculation of CHIKV and DENV could have great implication for human health. Interestingly, DENV is still circulating in the Caribbean, together with CHIKV. Cases of DENV-CHIKV coinfection in patients were first reported in 1967 (54), and since the emergence of CHIKV, reports of coinfections have been increasing (10, 55–63). Both viruses are transmitted by the same

mosquito vectors, *A. aegypti* and *A. albopictus*. Coinfection of a mosquito vector by two viruses can occur after two successive infectious blood meals taken from two different viremic hosts or after a single blood meal taken from a coinfecting host. It has been shown that CHIKV and DENV can be delivered together in one mosquito bite (64). As coinfections are a quite common phenomenon, consequences for the clinical presentation of the disease are expected.

Finally, the assessment of vector competence should be considered a prerequisite to better evaluate the potential risk of CHIKV outbreaks once the virus is introduced from regions of endemicity. The numerous imported CHIKV viremic cases presaged the potential importance of this emerging arbovirus for the Americas, where both mosquito species are well established. In light of epidemics now starting in the Caribbean, it remains imperative to pursue and reinforce epidemiological and entomological surveillance actions and control against mosquitoes of the species *A. aegypti* and *A. albopictus*.

ACKNOWLEDGMENTS

We thank Anayansi C Valderrama, Carlos Eduardo Borda, Darío Vezzani, Daniel Sánchez-Guillén, Eduardo A. Lestani, Fátima Domingos, Gabriel Sylvestre, Gabriela Willat, Glenda Velásquez, Ima Braga, José Bento Lima, L. Phil Lounibos, Lorenzo Carceres, Marcelo Celestino dos Santos, Márcia Gonçalves de Castro, Maria de Lourdes Macoris, Mário Navarro, Nidia Martínez Acosta, Maria C. Carrasquilla, Maria Inez L. Bersot, Mirian G. Palomino Salcedo, Mirko Rojas-Cortez, Oscar D. Salomon, Rafael Maciel-de-Freitas, Romeo Humberto Montoya, Sérgio L. Bessa Luz, Steve Juliano, Tamara Chávez Espada, Teresa F. Silva-do-Nascimento, and Yasmin Rubio-Palis for help with mosquito sampling in the field and/or mosquito rearing; Marie Vazeille and Laurence Mousson for technical advice; Myrielle Dupont-Pouzeyrol for providing CHIKV_{NC}; and Peter Sahllins and Henri Jupille for correcting the manuscript.

This work was funded by CNPq (grants 202106/2011-0 and 306340/2009-7), Instituto Oswaldo Cruz, Institut Pasteur (ACIP grant A-03-2012), and the French Government's Investissement d'Avenir program, Laboratoire d'Excellence "Integrative Biology of Emerging Infectious Diseases" (grant no. ANR-10-LABX-62-IBEID). A.V.-R. was supported by the French Ministry of Superior Education and Research and K.Z. by the European Community's Seventh Framework Programme (FP7/2007-2013) under the project "VECTORE", EC grant agreement no. 261466, and the Foundation Inkermann (Fondation de France).

The authors declare that they have neither competing interests nor conflicts of interest related to this article.

R.L.-D.-O. and A.-B.F. conceived the study. R.L.-D.-O., A.V.-R., and K.Z. carried out experimental infections of mosquitoes and performed titration assays. A.V.-R., R.L.-D.-O., and A.-B.F. drafted the manuscript. K.Z. and R.G. helped to draft and to revise the manuscript. All authors read and approved the final version of the manuscript.

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